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Basic Investigation

Effects of Bushen Huoxue Yin (补肾活血饮) on brain NF-κB and NO content in the parkinson's disease model mouse

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Abstract

OBJECTIVE: To observe the effects of Bushen Huoxue Yin (补肾活血饮, BSHXY) on nuclear transcription factor kappa B (NF-κB) and nitric oxide (NO) in the brain of the Parkinson's disease (PD) model mouse.

METHODS: Forty-five C57BL/6 mice were randomly divided into three groups; normal, model and BSHXY treatment groups. Concentrations of NF-κB and NO in mouse brain tissue were determined by ELISA and spectrophotometry, respectively.

RESULTS: NF-κB concentration in brain tissue in the model group was 14.04±4.38 μg·L^{-1}, which was higher than that in normal (P<0.01) and BSHXY (P<0.05) groups. NO content in brain tissue in the model group was 5.93±0.79 μmol·gprot^{-1}, which was also higher than that in model (P<0.01) and BSHXY (P<0.01) groups. However, there were no significant differences in the content of NF-κB and NO between BSHXY and normal groups (P>0.05).

CONCLUSION: The mechanism of BSHXY for treatment of PD is possibly related to inhibition of NF-κB activation and decreased NO content in the brain.

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Key words: Parkinson's disease; Bushen Huoxue Yin; Therapy of Traditional Chinese Medicine; Nuclear transcription factor kappa B; Nitric oxide

INTRODUCTION

Parkinson's disease (PD) is a retrograde disease of the central nervous system, which is clinically characterized mainly by static tremor, bradykinesia, muscular rigidity and abnormal posture. Presently, definite causes of PD are unclear and without specific therapeutic approaches. Therapy using a dopamine preparation shows obvious side effects, and after long-term administration, the therapeutic effect gradually decreases and even fails[1].Past studies indicate that Bushen Huoxue Yin (补肾活血饮, BSHXY) shows beneficial therapeutic effects, significantly improves symptoms and allows reduced dosages of Western medicines, thereby relieving their side effects[2,3]. In addition, BSHXY treatment increases the sensitivity of the dopamine receptor and promotes release of the dopamine transport protein in the striatum[4,5] to effectively treat PD. In this study, changes in nuclear transcription factor kappa B (NF-κB) and nitric oxide (NO) content in the brain tissue of the PD model mouse after administration of BSHXY were observed to investigate the possible mechanism of PD treatment.

MATERIALS AND METHODS

Experimental animals

Forty-five adult male C57BL/6 mice, sanitary degree, aged 8 – 10 weeks, weighing 20±2 g, were supplied by

the Experimental Animals Center, PLA General Hospital (Beijing, China) and maintained in a clean animal house at room temperature (25 ± 2°C) in 40% – 50% relative humidity with a 12 h light-dark cycle. Mice were allowed free access to water and food.

Main Reagents

1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) was purchased from Sigma Co. USA. Chloral hydrate solution (10%) was prepared by the Pharmaceutical Department of PLA general Hospital. NF-κB and NO kits were supplied by Beijing Zhongshan Biotechnique Co. Ltd (Beijing, China).

BSHXY was composed of Rou Cong Rong (Herba Cistanchis), Shan Yu Rou (Fructus Corni), Dang Gui (Radix Angelicae Sinensis), He Shou Wu (Radix Polygoni Multiflori), Chi Shao (Radix Paeoniae Rubra), Chuan Xiong (Rhizoma Chuanxiong), Wu Gong (Scolopendra) and Shi Chang Pu (Rhizoma Acori Graminei), and supplied and indentified by the Pharmaceutical Department of PLA General Hospital. Herbs were decocted by a traditional decoction method and concentrated in a water-bath at a constant temperature as a decoction containing the crude drugs at 1g • mL^{-1}. After disinfection, the decoction was poured into a sealed glass bottle and stored at 4°C until use[5]. The BSHXY dose for mice was 20g • kg^{-1} according to the calculation of the dose coefficient of experimental animals and humans.

Grouping of animals

Mice were randomly divided into three groups, normal, model and BSHXY groups, with 15 mice in each group.

Treatment of the animals[6]

Mice in the normal group were intragastrically injected with saline once a day for 7 days. The model group was intraperitoneally injected with MPTP (30 mg • kg^{-1}, prepared in saline) each day for 7 days, and were intragastrically injected with saline 1 h before the intraperitoneal injection of MPTP in the same volume as that used for the normal group. Mice in the BSHXY group were intraperitoneally injected with MPTP each day for 7 days at the same dose used for the model group, and were intragastrically injected with the BSHXY concentrated solution at the same volume used for the normal group at 1 h before intraperitoneal MPTP injection. For mice in the model group, approximately 0.5 h after the first intraperitoneal MPTP injection, short-term excitation and then general vibration, erect hair, tail raising and after the last injection, reduction of activity, walking haltingly and slower movement occurred, which in combination with the results of climbing pole [7] and hang [8]tests were used to judge modeling success.

Mice were sacrificed by decapitation at 6 h after the last injection on day 7, and then brain tissues were harvested[9].

Detection of NF-κB content

NF-κB content in brain homogenates was detected with an ELISA according to the manufacturer's instructions. After sacrifice, 0.5 g left brain tissue was harvested and placed on an ice-cold plate, homogenized with 500 μL saline and then centrifuged at 4000 r • min^{-1} for 10 min. The supernatant was stored at – 80°C. Fifty microliters of standard solutions, 50 μL samples and 50 μL distilled water were respectively added into blank microwells in order in an enzyme-labeled plate coated by an anti-NF-κB specific antibody. One hundred microliters of enzyme-labeled solution was added to each well except for the control well, then sealed with sealing gel, incubated at 37°C for 1 h, fully rinsed with washing solution three times and then dried with filter paper. Fifty microliters each of color reagents A and B were added to each well except for the control well, followed by incubation in a dark box at 37°C for 15 min, and then 50 μL stop solution was added to each well. OD values were detected by a microplate reader at 450 nm within 50 min, and a standard curve was prepared with the OD values as the vertical ordinate and the standard solution concentrations as the horizontal ordinate. The concentrations of samples were calculated based on the standard curve according to the sample OD, and the sensitivity was 1.0 μg • L^{-1}.

Detection of NO content

Right brain tissue was harvest and placed on an ice-cold plate, rinsed with ice-cold saline and then dried with filter paper. 500 milligrams of brain tissue was collected and 4.5 mL cold saline was added to prepare a 10% homogenate that was centrifuged at 2000 r • min^{-1} for 10 min. The supernatant was collected and centrifuged again at 10000 r • min^{-1} for 15 min. Then, the supernatant was kept for determination of NO content with spectrophotometry according to the manufacturer's instructions.

Statistical analysis

SPSS 11.5 statistical software was used for statistical analysis. Data were expressed as the mean±standard deviation ($\bar{x} \pm s$), and one-way analysis of variance was used for comparison among groups.

RESULTS

Comparison of NF-κB content in brain tissue homogenates among groups

Table1 Comparison of NF-KB content in brain tissue homogenates among groups ($\bar{x} \pm s$)

Group	n	NF-κB(μg·L^{-1})
Normal	15	8.93±2.25
Model	15	14.04±4.38▲
BSHXY	15	10.91±3.82*

Notes: ▲P<0.01 vs. normal group; *P<0.05 vs. model group.

Table1 Comparison of NO content in brain tissue homogenates among groups($\bar{x} \pm s$)

Group	n	NO(μmol·gprot^{-1})
Normal	15	3.27±0.66
Model	15	5.93±0.79▲
BSHXY	15	3.91±0.86*

Note: ▲P<0.01 vs. normal group; *P<0.01 vs. model group.

As shown in Table 1, NF-κB content in brain tissue homogenates was the lowest in the normal group and the highest in the model group, with significant differences between normal and model groups (P<0.01) and between BSHXY and model groups (P<0.05). No significant difference was found between BSHXY and normal groups (P>0.05).

Comparison of NO content in brain tissue homogenates among groups

As shown in Table 1, NO content in brain tissue homogenates was the lowest in the normal group and the highest in the model group, with significant differences between normal and model groups (P<0.01), and between BSHXY and model groups (P<0.05). No significant difference was found between BSHXY and normal groups (P>0.05).

DISCUSSION

The main pathological characteristic of PD is involved in the nigro-striatal system. MPTP is a neurotoxin that induces degeneration of dopaminergic neurons in the nigro-striatal system of the C57BL/6 mouse. The compound is highly fat-soluble and very easily passes through the blood-brain barrier and converts to 1-methyl-4-phenylpyridinium (MPP$^+$) under the action of monoamine oxidase to selectively injure dopaminergic neurons in the substantia nigra, hence the mouse produces symptoms very similar to those of PD[10]. The C57BL/6 PD mouse model prepared by MPTP is presently one of the generally recognized PD animal models at home and abroad, and has been widely used to study the pathogenesis and treatment of PD[11].

PD is a commonly observed retrograde disease of the brain with an unclear cause thus far. Progressive degeneration, necrosis and loss of dopaminergic neurons in the nigro-striatal system, proliferation of gliacytes and formation of Lewis corpuscle are the main pathological changes in PD[1]. Presently, its pathogenesis is unclear, but is possibly related to factors such as genetic inheritance, the action of environmental factors, oxidation stress, excitatory neurotoxin action and immuno-inflammatory responses. In addition, previous studies indicate that NF-κB and NO play a very important role in the development of PD[12,13].

NF-κB is a group of transcription factors in the Rel protein family and are present in the form of homologous or heterologous complexes. In the resting state, NF-κB protein is localized in the cytoplasm and combines with inhibitor kappa B (IκB) in a non-active state. NF-κB can be activated by specific cytokines, protein kinases, oxidizing agents, viruses, lipopolysaccharides and ultraviolet radiation[14]. In the central nervous system, NF-κB is widely expressed and activated by numerous neurotrophic factors, cytokines and cytotoxins. Activated NF-κB can induce the expression of cytokines, growth factors, acute proteins, chemotactic factors, immuno-receptors and transcription factors, which participate in various pathological courses of immune and inflammatory responses of diseases[15]. Autopsy of PD patients indicates increased NF-κB expression in the nucleus of dopaminergic neurons in the substantia nigra[16]. The results of the present study indicated that NF-κB content in the model group was significantly higher than that in the normal group (P<0.01), suggesting that NF-κB in the brain tissue of the model group was activated. After treatment with BSHXY in the BSHXY group, NF-κB content was not significantly higher compared with that in the normal group (P> 0.05), but was significantly higher than that in the model group (P<0.05), indicating that BSHXY decreases the expression of harmful factors possibly via inhibition of NF-κB activation to treat PD.

NO is a small signaling molecule with broad biological effects and is produced from the de-guanidinium group of L-arginine, catalyzed by inducible nitric oxide synthase (iNOS) in the body in a normal state and is sustained in a dynamic balance in tissues[17]. Almost all neural cells in the brain are able to synthesize NO, but excessive NO can cause toxicity-induced cell injury. A previous study indicated that high levels of NO play an important role in the pathogenic mechanisms of PD[13]. NO also can disrupt membrane structures and cause dysfunction of cell and mitochondrial membranes, leading to neural cell death. In addition, NO acts on neural cell DNA by introducing breakages in single- and double-stranded DNA followed by cross-linking that leads to defects and erroneous pairing of DNA bas-

es resulting in cellular necrosis and apoptosis. It can also induce neural cell injury via activating ADP-ribose synthase (PARS) and then by the energy-consuming reaction of PARS. NO itself as a free radial shows cellular toxicity that induces cell injury. In addition, it can interfere with the energy metabolism of cells and initiate apoptosis. From these observations, it can be inferred that NO is involved in DA neuron injury via multiple pathways. In this study, we showed that NO content in the model group was significantly higher than that in the normal group (P<0.05), indicating that a large amount of NO is produced in the brain tissue of the model group. In the BSHXY group, NO content was similar to that of the normal group (P> 0.05) and significantly lower than that in the model group (P<0.05), suggesting that BSHXY treats PD possibly via decreasing NO content and reducing neural toxicity.

Previous studies found that BSHXY shows beneficial effects on PD patients and promotes cellular repair in the substantia nigra and increases dopaminergic neuron activity in PD model animals[18,19]. These effects may possibly occur via inhibition of NF-κB activation and decreased NO content in the brain, which protects neurons from injury, thereby effectively treating PD. NF-κB is closely associated with iNOS, a rate-limited enzyme of NO synthesis, and regulates transcription of iNOS mRNA. NO, a product of iNOS, has a feedback inhibitory action on the activation of NF-κB[20]. However, in this study, NF-κB and NO content in the mouse brain of the model group increased, but decreased respectively after treatment with BSHXY, thus the interaction between them requires further study.

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